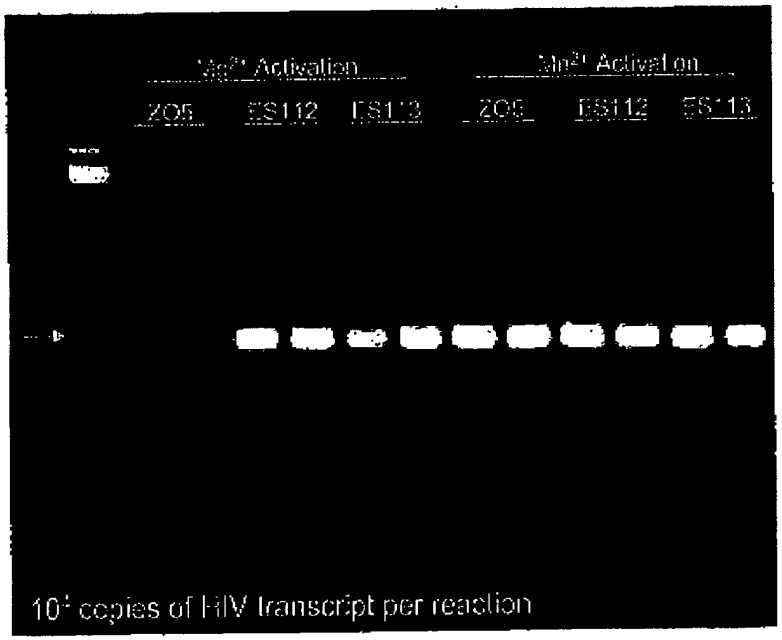
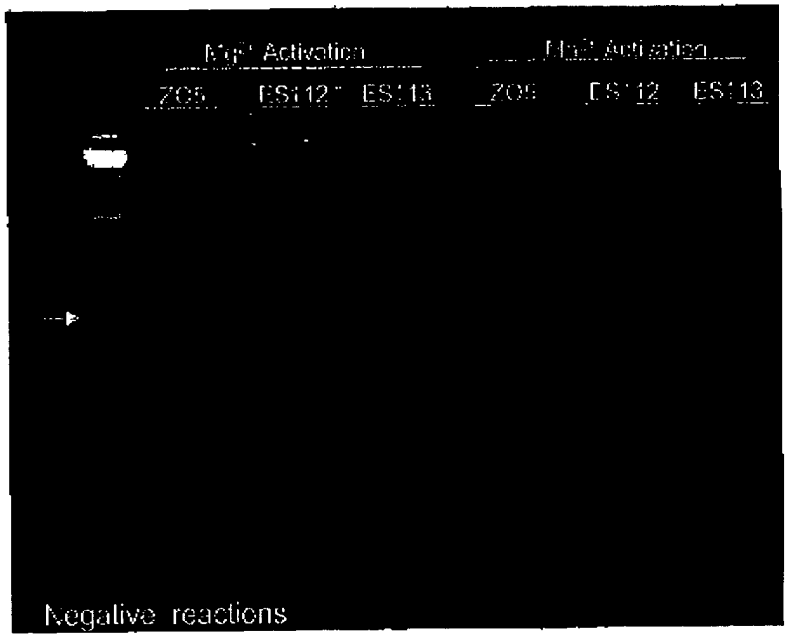
012/025

Improved Mg^{2+}-activated RT-PCR with ES112 & ES113





Three different thermostable DNA polymerases were used to reverse transcribe an HIV transcript RNA template and subsequently amplify the cDNA in a coupled RT-PCR in the presence of either 3 mM Mg^{2+} or 3 mM Mn^{2+}. After 55 cycles of PCR, gel results demonstrate specific amplification products from RNA with ZO5 in the presence of Mn^{2+}, but no specific product was observed when Mg^{2+} was used as the divalent metal ion activator. However, designer enzymes ES112 and ES113 produced specific amplification product with either Mg^{2+} or Mn^{2+} activation.

05/05/2004 13:00 FAX 415 576 0300

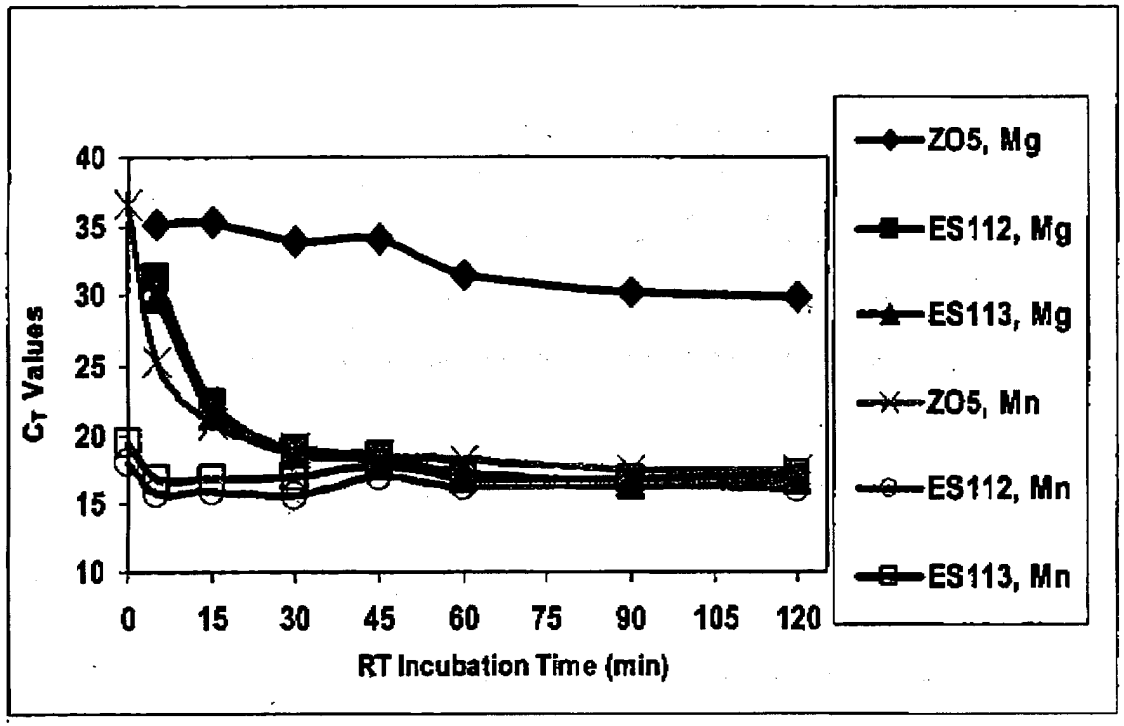
PAGE 12/25 * RCVD AT 5/5/2004 3:55:11 PM [Eastern Daylight Time] * SVR:USPTO-EFXRF-3/24 * DNIS:2730743 * CSID:415 576 0300 * DURATION (mm-ss):09-38

PAGE 13/25 * RCVD AT 5/5/2004 3:55:11 PM [Eastern Daylight Time] * SVR:USPTO-EFXRF-3/24 * DNIS:2730743 * CSID:415 576 0300 * DURATION (mm-ss):09-38

05/05/2004 13:00 FAX 415 576 0300

Reduced RT Time Requirement for ES112 & ES113 in Mn^{2+}

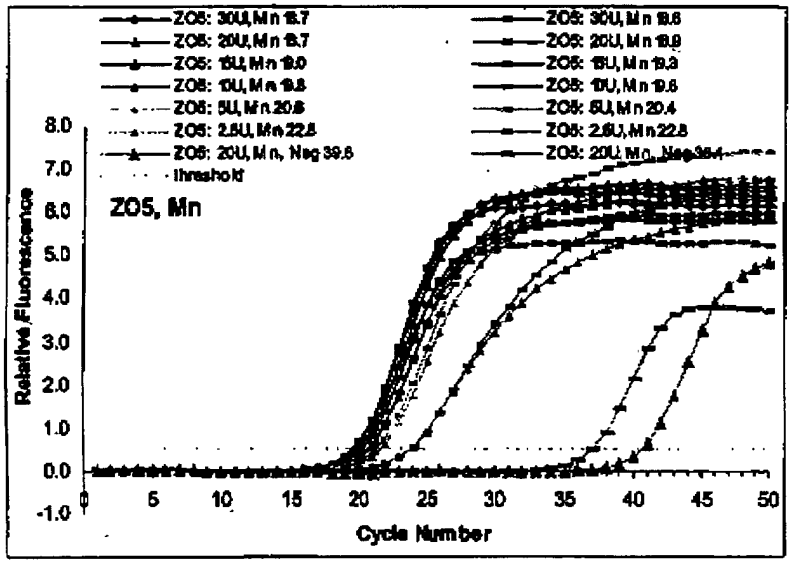
A 280 bp GAPDH RNA template was subjected to various RT incubation times and then amplified by PCR. In all cases PCR profiles were identical and the results were analyzed by kinetic PCR. The C_T values of growth curves are plotted in the following chart:

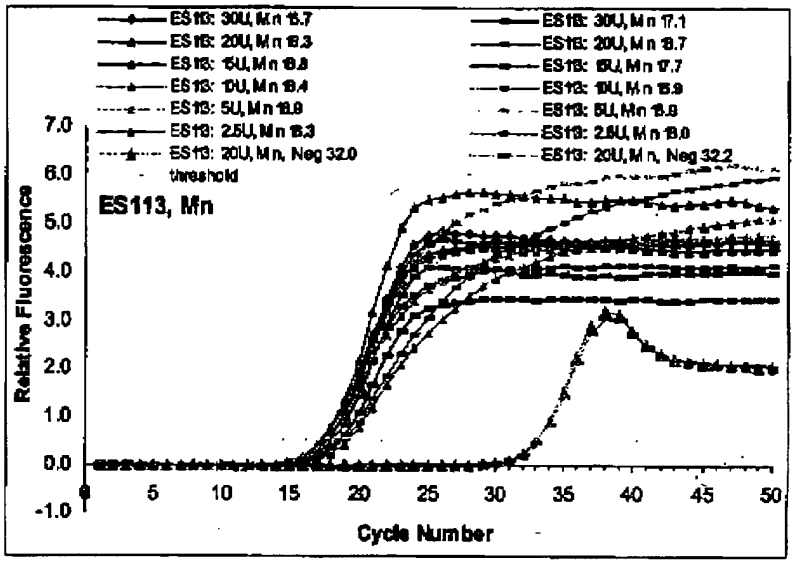


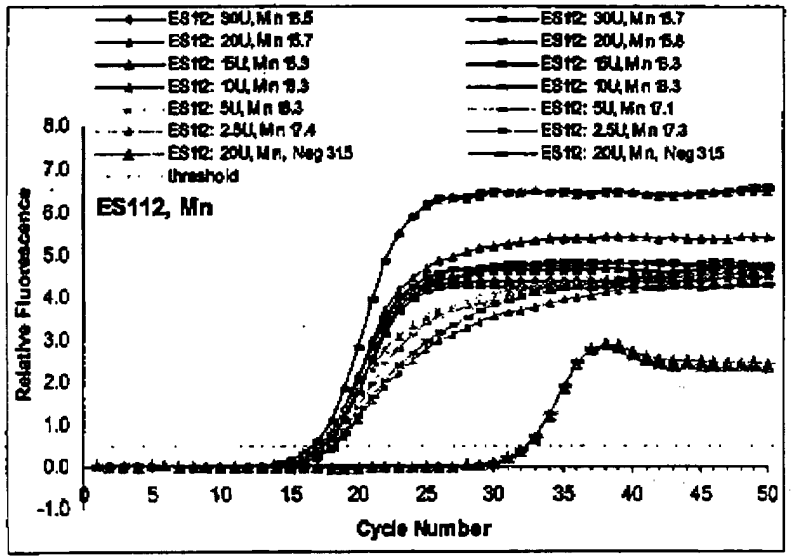
Following a 30 min RT incubation time and Mg^{2+} activation, the mutant enzymes ES112 and ES113 achieved RT activity similar to Mn^{2+}-activated wild-type ZO5 DNA polymerase. With Mn^{2+} activation, the mutant enzymes exhibited similar RT activity, but with much shorter RT incubation times (as low as 5 min). Even with no added RT incubation time there were only slight C_T delays for Mn^{2+}-activated mutant enzyme amplifications and initial PCR ramp times apparently are sufficient for the RT step to occur.

013/025

Efficient RT-PCR at Decreased ES112 & ES113 Enzyme Concentrations



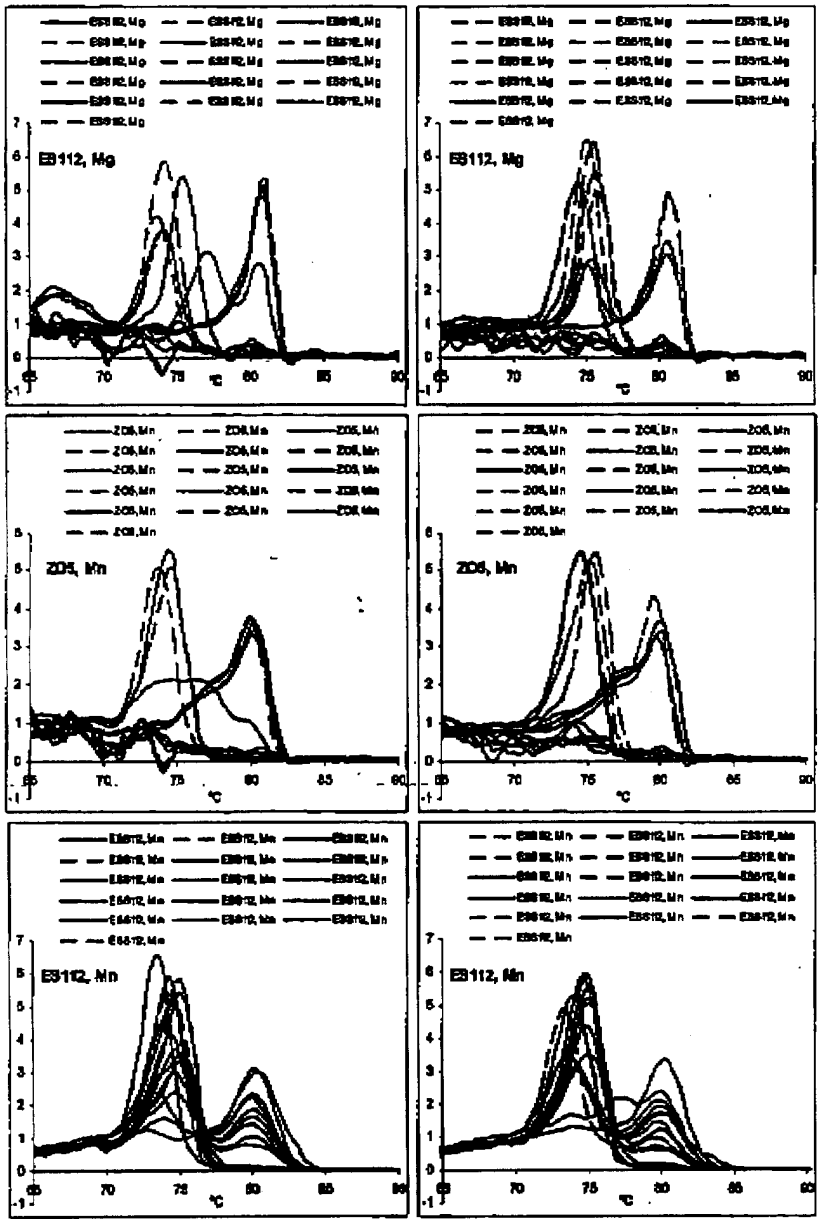




Enzyme concentration was titrated from 30 U down to 2.5 U per reaction for ZO5, ES112 and ES113. A significantly higher C_T value is observed with 2.5 U of ZO5 when compared to higher enzyme concentrations. The ES112 and ES113 perform relatively efficient RT-PCR with as little as 2.5 U of enzyme per 50 µL reaction.

015/025

Improved Low Copy Sensitivity with ES112 in Mn^{2+}-activated RT-PCR



ES112, Mg^{2+}

10/32 Positives

ZO5, Mn^{2+}

10/32 Positives

ES112, Mn^{2+}

20/32 Positives

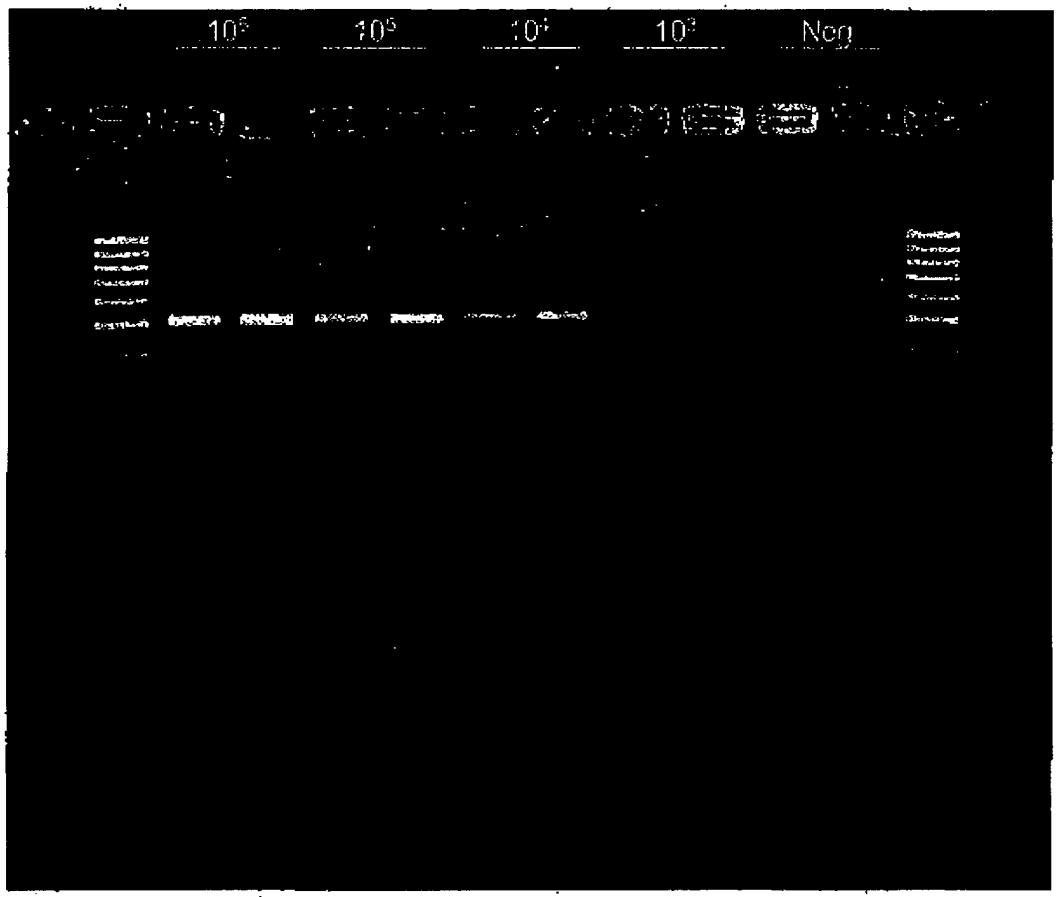
Nominally 0.5 copies of HIV transcript RNA per reaction were amplified in 50 µL RT-PCR amplifications optimized for Mg^{2+}-activated ES112, Mn^{2+}-activated ES112 or Mn^{2+}-activated ZO5 ("Gold Standard"). The T_m of end-point RT-PCR product was used to distinguish successful amplification of transcript RNA (specific product) from negative reactions (nonspecific product). The Mg^{2+}-activated ES112 reactions had the same low copy sensitivity as the Mn^{2+}-activated ZO5, while the low copy sensitivity was observed to be twice as good with Mn^{2+}-activated ES112.

05/05/2004 13:00 FAX 415 576 0300

PAGE 15/25 * RCVD AT 5/5/2004 3:55:11 PM [Eastern Daylight Time] * SVR:USPTO-EFXRF-3/24 * DNIS:2730743 * CSID:415 576 0300 * DURATION (mm-ss):09-38

016/025

RT-PCR Using Mg^{2+}-activated CS6 DNA Polymerase



Various concentrations of pAW109 transcript RNA were amplified by single-buffer RT-PCR. All reactions contained 2 mM Mg^{2+} and CS6 DNA polymerase. Following 45 cycles of PCR, products of the correct size were observed with as little as 10^3 copies of RNA per reaction. Negative control reactions lacking RNA transcript produced no specific product of the expected amplicon size.

05/05/2004 13:01 FAX 415 576 0300

PAGE 16/25 * RCVD AT 5/5/2004 3:55:11 PM [Eastern Daylight Time] * SVR:USPTO-EFXRF-3/24 * DNIS:2730743 * CSID:415 576 0300 * DURATION (mm-ss):09-38